HIGH LINOLENIC ACID FLAX

FIELD OF THE INVENTION

The present invention relates generally to the field of agriculture. More specifically, the present invention relates to flax seeds, plants and oils having altered fatty acid profiles.

per paper #3

BACKGROUND OF THE INVENTION

Unsaturated fatty acids consist of monosaturates and polyunsaturates (PuFA). There are two classes of PuFA's, omega-3 and omega-6 which are considered as essential fatty acids (EFA) because humans, like all mammals, cannot synthesize them and most obtain them in their diet. Omega-3 is represented by alpha-linolenic acid (LNA) and linseed flax has the highest linolenic content of major seed oils. LNA is metabolized to eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA), increasing the chain length and degree of unsaturation by adding double bonds to the carboxyl group. Humans and animals can convert LNA to EPA and DHA.

Essential fatty acids have several vital functions: they increase metabolic rate, improve metabolism, increase oxygen uptake and increase energy production. EPA and their derivatives are components of membranes surrounding cells; they are required for the transport and metabolism of cholesterol and triglycerides; they are required for normal development of the brain and for brain function in adults; and from EPA's the body makes hormone-like substances called prostaglandins which have important regulating functions in the body, i.e. they regulate arterial muscle tone,

sodium excretion through the kidneys, platelet stickness, inflammatory response and immune functions.

In addition to being essential for normal growth and development, omega-3 fatty acids may play an important role in the prevention and treatment of coronary artery disease, hypertension, diabetes, arthritis, and other inflammatory and anti-immune diseases, and cancer.

Linseed flax is grown primarily for its oil which has many industrial uses; manufacture of paints and coatings, oil cloth, printing ink, soap, patent leather, core oils, brake linings, etc. Linseed oil is also used as an anti-spalling and curing agent for concrete surfaces including highways and bridges.

Linseed oil is used as an industrial oil because of its drying property, i.e. ability to dry rapidly to form a durable film upon exposure to air. The drying property of linseed oil is due to the high content of unsaturated fatty acids, particularly linolenic acid. The linolenic content of Canadian flax cultivars varies from 49-62% depending on cultivar and growing conditions. Temperature during seed formation and photoperiod influences the degree of unsaturation of the fatty acid in linseed oil. Cool temperatures and longer photoperiod increases linolenic content. Other factors which may affect the variability of linolenic content are soil moisture, soil fertility and presence of disease.

High linolenic flax oil provides a valuable improved source of omega-3 fatty acid for human nutrition, animal feed and pet foods.

Higher linolenic content of linseed oil is desirable for most of the industrial uses and may result in new industrial uses.

Clearly, there is a need for high linolenic acid flax.

SUMMARY OF THE INVENTION

According to a first aspect of the invention, there is provided a flax seed having a linolenic acid content of greater than 65% of the total fatty acid content of said seed.

According to a second aspect of the invention, there is provided a flax seed that is the product of a plant line designated M5791.

According to a third aspect of the invention, there is provided a flax plant which produces seeds having a linolenic acid content of greater than 65% of the total fatty acid content of said seed.

According to a fourth aspect of the invention, there is provided a flax plant designated M5791.

According to a fifth aspect of the invention, there is provided progeny of a flax plant designated M5791, wherein said progeny produce seeds having a linolenic acid content of greater than 65% of the total fatty acid content of said seed.

According to a sixth aspect of the invention, there are provided seeds from the above-described flax plants.

According to a seventh aspect of the invention, there is provided oil manufactured from the seeds.

According to an eighth aspect of the invention, there is provided a method of producing a flax plant line comprising the steps of:

(a) crossing a plant of a flax plant line designated M5791, or progeny thereof, with an agronomically elite flax plant;

(b) selecting at least one decendant of said cross, said decendant producing seeds having a linolenic acid content of greater than 65% relative to the total fatty acid content of said seed,

(c) the flax plant is self-pollinated and, therefore, the linolenic content of seed progeny of M 5791 is stable.

BRIEF DESCRIPTION OF THE DRAWINGS

TABLE 1 shows the fatty acid composition of major seed oils.

TABLE 2 shows fatty acid composition and iodine value of linseed flax and high linolenic flax (M 5791).

TABLE 3 shows the linolenic acid content of Canadian Flaxseed by province.

TABLE 4 shows linolenic acid content of four widely grown Canadian flax cultivars in flax co-operative tests.

TABLE 5 shows the analysis of linolenic acid content of M5791.

TABLE 6 shows the linolenic acid content of M5791 in field scale tests.

FIGURE 1 shows the structural formula for fatty acids.

FIGURE 2 shows the elongation and desaturation of n-6 and n-3 polyunsaturated fatty acids.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to

which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned hereunder are incorporated herein by reference.

DEFINITIONS

As used herein, “high linolenic flax” refers to flax seeds or plants wherein the percentage of total fatty acid content that is linolenic acid is greater than 65%.

As used herein, “inflammatory disease” refers to diseases that are associated with an inappropriate or over-activation of the inflammation cascade.

As used herein, “autoimmune disorder” refers to diseases characterized by destruction of host tissue by the host immune system.

The invention relates to flax seeds, plants or products made therefrom wherein linolenic acid is greater than 65% of the total fatty acid content. In some embodiments, the linolenic acid content may be greater than 70% of the total fatty acid content. In yet other embodiments, the linolenic acid content may be 70-80% or 70-75% of the total fatty acid content.

As discussed herein, the crossing of flax lines has produced a high linolenic flax line, designated M5791. As shown in Tables 4 and 5, M5791 shows much higher linolenic acid content relative to other flax lines.

As will be appreciated by one knowledgeable in the art, and as discussed herein, growth conditions can affect linolenic acid content. However, flax line M5791 can be grown and processed using means known in the art for growing and processing flax.

The linolenic content of M 5791 oil is not altered by any methods of extraction of oil from seed, i.e. solvent, cold press, or distillation.

As discussed above, flax and/or linseed oil have are present in a number of industrial, nutritional and medicinal products.

For example, linseed oil is used as an industrial oil because of its drying properties, which are in turn directly proportional to the linolenic acid content. Industrial uses of this oil include but are by no means limited to the manufacture of brake linings, adhesives, manufacture of hardboard and fibreboard, protective coatings, paints, house paint primers, varnishes, lacquers, stains, alkyd resins, enamels, epoxidized oils, floor coverings, linoleum, oilcloth, tarpaulin and other coated fabrics, patent leather, industrial chemical, fatty acids, soap, glycerin, printing inks, grinding oils, newsprint, core oils, caulking compounds, waterproofing compounds, mastic cements, foundry binders, brake lining, hardboard, shoe polish, herbicide, pesticide carrier, antispalling and curing treatments for concrete, tempering oil, bonding oil, and highly conjugated oils for hardboards and the like. Thus, use of linseed oil from high linolenic acid flax will result in, for examples, faster drying paints, coatings, inks and the like. Examples of industrial uses of linseed oil include but are by no means limited to US Patents 5,965,633, 5,693,715, 5,653,789, 3,488,202 and 4,002,585.

Furthermore, the meal remaining from the flax seed oil extraction processes described above also has industrial purposes, for example, in animal feed or pet food. As will be appreciated by one knowledgeable in the art, meal from the high linolenic acid flax seeds may be used in any application known in the art for using meal

from flax seeds, wherein the meal has the added benefit of having a higher linolenic acid content.

The above-described flax seed may be used, for example, to produce high protein animal feed. For example, the seed, oil, or meal may be used in feed for dairy and beef cattle, swine and/or poultry to increase levels of linolenic acid in the resulting food products, for example, eggs, meat and milk. As discussed above, the seed and meal may also be used in pet foods. The seed and oil may also be used as a source of linolenic acid for growing fish.

As discussed above, polyunsaturated fats, for example, linolenic acid are essential fatty acids and are required for increased metabolism, oxygen uptake, and energy production. As such, nutritional supplements or food products containing material produced or processed from the high linolenic acid flax line would be higher in polyunsaturated fats and therefore of greater benefit. As such, the high linolenic flax line, seeds or products derived therefrom can be used in, for example, nutritional supplements, food products for human consumption, pet foods and animal feeds. Examples of edible products include, but are by no means limited to, cakes, muffins, bread products (whole seed or flour), replacement for sesame seed in baking products, or cooked and dry cereals Examples of the use of linolenic acid or flax products in nutritional supplements or food products include but are by no means limited to US Patent 5,069,903 and 6,060,101.

As discussed above, linolenic acid also plays a role in the prevention and/or treatment of a number of diseases, including , for example, coronary artery disease, hypertension, diabetes, arthritis and other inflammatory or autoimmune

disorders. In these embodiments, supplements or pharmaceutical compositions including material derived from the high linolenic acid flax are prepared and used to treat and/or prevent the above-referenced diseases or disorders. Examples of medicinal uses for flax and/or linseed oil include but are by no means limited to US Patents 5,859,055, 4,415,554, 5,468,776 and 4,058,594.

In these embodiments, the high linolenic linseed oil or high linolenic flax may be included in a tablet, capsule, tincture, salve, paste, cream or the like.

In some embodiments, the high linolenic acid flax or linseed oil therefrom at therapeutically effective concentrations or dosages may be combined with a pharmaceutically or pharmacologically acceptable carrier, excipient or diluent, either biodegradable or non-biodegradable. Exemplary examples of carriers include, but are by no means limited to, for example, poly(ethylene-vinyl acetate), copolymers of lactic acid and glycolic acid, poly(lactic acid), gelatin, collagen matrices, polysaccharides, poly(D,L lactide), poly(malic acid), poly(caprolactone), celluloses, albumin, starch, casein, dextran, polyesters, ethanol, mathacrylate, polyurethane, polyethylene, vinyl polymers, glycols, mixtures thereof and the like. Standard excipients include gelatin, casein, lecithin, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glyceryl monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, polyoxyethylene stearates, colloidol silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethycellulose phthalate,

noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, polyvinylpyrrolidone, sugars and starches. See, for example, Remington: The Science and Practice of Pharmacy (20th Edition), 2000, Gennaro, AR ed., Eaton, PA: Mack Publishing Co.

In yet other embodiments, oil from the seeds of the high linolenic acid flax plants are used as carriers or diluents for other pharmaceutical or medicinal compounds.

As will be apparent to one knowledgeable in the art, specific carriers and carrier combinations known in the art may be selected based on their properties and release characteristics in view of the intended use.

Thus, the high linolenic acid flax line, seeds therefrom or products derived therefrom can be used in the production of any flax-containing products known in the art. As will be appreciated by one knowledgeable in the art, these products will have the added benefit of having a higher linolenic acid content.

As will be appreciated by one knowledgeable in the art, the flax plant designated M5791 can be used to perform genetic crosses with other flax lines using means known in the art.

The invention will now be described by way of examples, although the invention is in no way limited to the examples.

EXAMPLE I – BREEDING METHOD

Breeding Method

High Linolenic flax was developed by conventional plant breeding, ie. hybridization using cultivars, breeding lines and accessions, and selections for high linolenic acid content in segregating populations from crosses, at the AAFC Morden Research Centre, Morden, MB. The development of High Linolenic flax involved 6 crosses. In each of the crosses, the F2 and subsequent generations of crosses were advanced to the F7 generation using the pedigree method of breeding. Selection for high linolenic was initiated in the F2 generation. F2 plants were analyzed on a half-seed basis by gas-liquid chromatography of the fatty acid esters using the method described by Daun et al, J. Amer. Oil Chemists' Society, 60, 1983. Using the remainder of the seed, the F3 generation of high linolenic genotypes were grown in the greenhouse. Single plant selections were made in the F4 generation on basis of linolenic content. The F5 generation of selected lines was grown in a winter nursery. F6 lines were selected for high linolenic content at Morden and seed harvested in bulk. Seed of F7 lines was increased in a winter nursery. High linolenic flax was evaluated in replicated trials conducted in 1998, 1999 and 2000, and in field trials conducted in 1998 and 1999 (Table 5).

Cross

F_1 - greenhouse

F_2 - field; high linolenic genotypes selected by ½ seed analysis

F_3 - greenhouse

F_4 - field; plants selected for high linolenic content

F_5 - winter nursery

F_6 - field; lines selected for high linolenic and seed harvested in bulk

F_7 - Seed increase in winter nursery

F_8 - Replicated trials in Manitoba

F_9 - Replicated trials in Manitoba and field trials in Manitoba and Alberta

93-14492/93-15117y/3/92-235-4/93-15117y

93-14492 - NorLin/5173 (M 2854)//Culbert/FP 827 (91-4086)

93-15117y - AC Linora/427-2 (91-9791)//NorLin/Szegedi 62 (91-4306)

92-235-4 - NorLin/5173 (M 2854)//McGregor/FP 842 (M 3547)

Date of crosses:

Crosses 1 & 2 - 1993

Cross 3 - 1995

Pedigree/origin of Parents in crosses:

Cultivars	Origin
AC Linora[1]	AAFC
AC McGregor[2]	AAFC
NorLin[3]	AAFC
Raja	AAFC
Redwood 65[4]	U. of Saskatchewan, Saskatoon, SK
Culbert[5]	U. of Minnesota, St. Paul, Minn.

Lines (developed by AAFC)

5173 - McGregor//Redwood 65/High Oil Line/3/CI 2941

FP 827 - McGregor/3/Redwood 65//Valuta/Raja

FP 842 - CI 2847/Culbert

Accessions	Origin
Szegedi 62	Hungary
Valuta	Sweden
CI 2847	USA
CI 2941	USA
427-2	Argentina
High Oil Line	Unknown

Description of Cultivars

[1] Can. J. Plant Sci. 73:839-841, 1983

[2] Can. J. Plant Sci. 66:175-176, 1986

[3] Can. J. Plant Sci. 66:171-173, 1986

[4] Can. J. Plant Sci. 45:515-516, 1965

[5] Crop Sci. 17:823, 1977

While the preferred embodiments of the invention have been described above, it will be recognized and understood that various modifications may be made therein, and the appended claims are intended to cover all such modifications which may fall within the spirit and scope of the invention.

Table 1. Fatty Acid Composition of Major Seed Oils

	Fatty Acid Content %			
	Saturates	Oleic	Linoleic	Linolenic
Linseed Flax	9	20	13	58
Canola	6	58	26	10
Safflower	9	20	70	<1
Sunflower	11	20	69	-
Corn	13	25	61	1
Olive	14	77	8	1
Soybean	15	24	54	7
Peanut	18	48	34	-
Cottonseed	27	19	54	-

Source: Agricultural Handbook No. 8-4. Human Nutr. Inform. Serv., U.S. Dept. Agric., Washington, DC. 1979.

Table 2. Fatty Acid Composition of Linseed Flax and High Linolenic Flax (M 5791)

Fatty acid, % in oil		Linseed Flax	High Linolenic Flax
Palmitic	C16:0	5.4	4.5
Stearic	C18:0	3.1	2.2
Oleic	C18:1	17.1	9.7
Linoleic	C18:2	14.7	10.8
Linolenic	C18:3	59.6	72.8
Iodine value		196	217

Table 3. Linolenic Acid Content of Canadian Flaxseed by Provinc - 1992-1999*

	Manitoba	Saskatchewan	Alberta	Mean
1992	59.1	58.7	55.7	58.8
1993	57.9	61.0	60.7	59.7
1994	58.6	60.5	56.9	59.6
1995	55.8	59.4	60.8	58.1
1996	57.8	59.3	59.8	58.7
1997	58.8	57.4	58.9	58.0
1998	57.2	56.6	56.9	56.8
1999	60.4	59.4	59.0	59.6
Mean	58.2	59.0	58.6	58.7

Source: Quality of Western Canadian flaxseed, Grain Research Laboratory, Canadian Grain Commission, Winnipeg, MB. Linolenic acid determined by gas chromatography of methyl esters of fatty acids according to International Organization for Standardization; Animal and vegetable fats and oils 1505508:1990E.

Table 4. Linolenic Acid Content of Four Widely Grown Canadian Flax Cultivars in Flax Cooperative Tests* conducted at Eight Locations in Manitoba and Saskatchewan, 1995-1999.

	Manitoba				Saskatchewan				
	Morden	Portage la Prairie	Rosebank	Brandon	Indian Head	Melfort	Saskatoon	Scott	Mean
1995									
AC McDuff	52.5	53.8	53.5	49.0	57.2	56.2	54.9	56.2	54.2
CDC Normandy	54.0	55.7	55.8	52.8	60.2	56.8	56.5	58.7	56.3
Flanders	55.0	56.5	56.3	52.0	60.2	58.9	57.5	58.9	56.[illegible]
NorLin	53.8	55.4	54.5	53.1	60.0	56.7	56.5	58.9	56.1
Mean	53.8	55.4	55.0	51.7	59.4	57.1	56.3	58.2	55.9
1996									
AC McDuff	53.6	55.2	55.5	52.6	55.4	53.8	54.4	56.0	54.6
CDC Normandy	55.8	54.6	57.6	54.9	58.2	56.7	56.4	58.1	56.5
Flanders	57.3	58.3	58.0	54.3	57.8	56.2	56.8	558.7	57.2
NorLin	55.2	54.2	56.8	54.7	57.8	55.4	56.0	57.7	56.0
Mean	55.5	55.6	57.0	54.1	57.3	55.5	55.9	57.6	56.0
1997									
AC McDuff	53.3	55.4	56.0	52.2	49.7	53.1	52.1	50.6	52.8
CDC Normandy	53.0	55.0	58.0	52.1	52.9	51.6	55.1	54.3	54.0
Flanders	55.9	58.9	58.1	53.8	53.9	54.8	55.1	53.7	55.[illegible]
NorLin	52.9	56.8	57.7	53.1	51.9	52.5	55.4	53.9	54.[illegible]
Mean	53.8	56.5	57.4	52.8	52.1	53.0	54.4	53.1	54.1
1998									
AC McDuff	51.5	51.9	51.4	46.9	50.8	47.9	49.5	45.5	49.5
CDC Normandy	53.8	53.2	53.2	48.9	53.7	51.4	52.3	48.2	51.8
Flanders	52.6	54.3	54.6	49.7	53.5	52.7	53.0	47.8	52.3
NorLin	52.7	53.5	54.0	49.8	53.4	50.0	51.3	48.0	51.6
Mean	52.6	53.2	53.3	48.8	52.8	50.7	51.5	47.4	51.3

Table 4 (Continued)

	Manitoba				Saskatchewan				
	Morden	Portage la Prairie	Rosebank	Brandon	Indian Head	Melfort	Saskatoon	Scott	Mean
1999									
AC McDuff	57.1	58.8	59.2	58.4	59.8	53.4	51.6	52.0	56.3
CDC Normandy	59.9	59.0	60.0	58.2	60.1	53.5	56.1	55.5	57.[illegible]
Flanders	60.2	61.1	62.4	49.0	61.4	54.6	54.7	55.6	58.7
NorLin	59.4	59.0	59.8	58.8	59.4	53.3	55.4	54.9	57.5
Mean	59.1	59.6	60.3	58.6	60.2	53.7	54.4	54.5	57.6
Overall Mean	55.0	56.0	56.6	53.2	56.4	54.0	54.5	54.2	

* Cooperative tests conducted by Prairie Registration Recommending Committee on Grain. Linolenic acid content determined by gas-liquid chromatography of the fatty acid esters using the method described by Daun et al., J. Amer. Oil Chemists' Society, 60, 1983.

Table 5. Linolenic Acid Cont nt* of High Linolenic Flax (M 5791) in Replicated Trials in Manitoba, 1998 & 1999, in Comparison with Three Commercial Cultivars

	Morden	Burdick	Portage la Prairie
1998			
High Linolenic Flax	70.1		
AC Emerson	57.0		
Flanders	53.1		
NorLin	55.2		
1999			
Test 1			
High Linolenic Flax	70.9	71.9	72.7
AC Emerson	61.7	60.6	65.1
Flanders	60.4	60.6	62.5
NorLin	59.7	57.8	59.3
Test 2			
High Linolenic Flax	71.9	70.3	
AC Emerson	58.6	59.2	
Flanders	58.2	60.3	
NorLin	56.9	59.3	
Test 3			
High Linolenic Flax	72.0	71.3	
AC Emerson	60.5	61.6	
Flanders	60.0	60.9	
NorLin	58.6	58.2	

* Linolenic acid content determined by gas-liquid chromatography of the fatty acid esters using the method described by Daun et al., J. Amer. Oil Chemists' Society, 60, 1983.

Table 6. Linolenic Acid Content of High Linolenic Flax (M 5791) in Field Trials, 1998 & 1999

Location	Year	% Linolenic Content
Fisher Branch, MB	1998	72.7
	1999	Field 1 72.7
		Field 2 72.2
Erickson, MB	1998	72.9
	1999	Field 1 72.9
		Field 2 72.9
Gadsby, AB	1998	72.6
	1999	Field 1 72.6
		Field 2 73.1

Uses of Flax

Linseed Oil (High Linolenic Acid)	Adhesives, i.e. manufacture of hardboard and fibreboard Protective Coatings: paints, house paint primers, varnishes, lacquers, stains, alkyd resins, enamels, epoxidized oils
Raw & Refined Boiled & Blown Grinding Oils Heat Boiled (polymerized oils)	Floor Covering: linoleum Oilcloth, tarpaulin and other coated fabrics Patent leather Industrial Chemicals: fatty acids, soap, glycerin Printing inks, grinding oils, newsprint, core oils, caulking compounds, waterproofing compounds, mastic cements, foundry binders, brake lining, hardboard, shoe polish, herbicide and pesticide carrier Antispalling and curing treatments for concrete Tempering oil, bonding oil, and highly conjugated oils for hardboards
Seed	Specialty Edible Products: - cakes, muffins - bread products (whole seed or flour) - replacement for sesame seed in baking products - cooked and dry cereals
Seed & Oil	Health Food: - source of linolenic acid - to make pills - inclusion in pharmaceutical mediator mutations
Linseed Meal	High protein animal feed Seed, oil, meal: Dairy and beef cattle, swine and poultry feed and also to increase levels of linolenic acid in food products, i.e. eggs, meat and milk Pet Foods: seed and meal Fish feed in fish culture: Seed and oil - source of linolenic acid required by growing fish, i.e. salmon